

What is Claimed:

1. A mammalian cell-based high throughput assay for the profiling and screening of putative modulators of an epithelial sodium channel (ENaC) comprising:

5        contacting a test cell expressing alpha, beta and gamma subunits or delta, beta and gamma subunits or a variant, fragment or functional equivalent of each of these three subunits and preloaded with a membrane potential fluorescent dye or a sodium fluorescent dye with at least one known ENaC inhibitor under conditions that at least partially inhibit ENaC function and  
10        thereafter contacting the test cell with at least one putative modulator compound in the presence of sodium or lithium; and

             monitoring anion mediated changes in fluorescence of the test cell in the presence of the putative modulator/ENaC interactions compared to changes in the absence of the modulator to determine the extent of ENaC  
15        modulation.

2. The method of Claim 1 wherein the known ENaC inhibitor is an amiloride derivative.

3. The method of Claim 2 wherein said compound is selected from the group consisting of Phenamil, benzamil, ethylisopropylamiloride; 2<sup>l</sup>, 4<sup>l</sup> –  
20        dimethylbenzamil (DMB), 5-(N-4-chlorobenzyl)-2<sup>l</sup>,4<sup>l</sup> – dimethylbenzamil (CBDMB); 3<sup>l</sup>, 4<sup>l</sup> – dichlorobenzamil; 5-(N-methyl-N-guanidinocarbonyl)methyl amiloride, 5-(N,N-hexamethylene)amiloride; 5(N-ethyl-N-isopropyl)amiloride (EIPA); 5-(N-4-chlorobenzyl)- 2<sup>l</sup>, 4<sup>l</sup> dimethylbenzamil; 2<sup>l</sup>, 4<sup>l</sup>; -dimethyl 2<sup>l</sup>, 3<sup>l</sup>-

benzamil 2<sup>l</sup>, 3<sup>l</sup>-benzobenzamil; and 5-(N-4-chlorobenzyl)- 2<sup>l</sup>, 4<sup>l</sup> dimethylbenzamil.

4. The method of Claim 3 wherein said compound is Phenamil.

5. The assay method of claim 1 in which is anion is sodium.

5 6. The assay method of claim 1 in which the anion is lithium.

7. The assay method of claim 1 in which the test cell is selected from the group consisting of MDCK, HEK293, HEK293 T, BHK, COS, NIH3T3, Swiss3T3 and CHO.

8. The assay method of claim 7 in which the cell is an HEK293 cell.

10 9. The a ssay method of claim 7 wherein said HEK293 cell is a n HEK293T cell.

10. The assay method of claim 1 in which a said method is used to identify a compound as one which particularly modulates taste based on a detectable change in fluorescence.

15 11. The assay method of claim 10 wherein said taste is salty taste.

12. The assay method of claim 1 in which said test cells are seeded onto a well of a multi-well test plate.

13. The assay method of claim 12 wherein said test cells are contacted with a putative modulator by adding said putative modulation to the  
20 well of said multi-well test plate.

14. The assay method of claim 13 wherein said test cells are loaded with a membrane potential dye that allows for changes in fluorescence to be detected.

15. The assay method of claim 14 wherein said test cell expresses each of the alpha, beta and gamma ENaC subunits.

16. The assay method of claim 15 wherein said subunits are respectively encoded by SEQ ID NO: 1, 2 and 3, or a fragment thereof, or a  
5 DNA sequence that hybridizes thereto and encodes a functional hENaC subunit.

17. The assay method of claim 1 wherein said subunits are encoded by SEQ ID NO: 1, 2 and 3.

18. The assay method of claim 1 wherein said test cell  
10 expresses hENaC beta, gamma and delta subunits or a fragment or variant thereof.

19. The assay method of claim 18 wherein said beta, gamma and delta subunits are respectively encoded by SEQ ID NO.: 2, 3 and 7.

20. The assay method of claim 1, wherein said ENaC subunits all  
15 comprise human ENaC subunits cloned from human kidney cDNA.

21. The assay method of claim 1, wherein said ENaC subunits comprise human ENaC subunits cloned from human lung cDNA.

22. The assay method of claim 1, wherein the ENaC is a human ENaC that is encoded by human ENaC DNA sequences cloned from human  
20 taste cell cDNA.

23. The assay of claim 1, wherein the ENaC is comprised of alpha (or delta), beta and gamma subunits and selected from the group consisting

of: a naturally occurring human ENaC, an alternatively spliced human ENaC, a functional variant thereof, or combinations thereof.

24. The assay of claim 1 wherein a fluorescence plate reader is used to monitor changes in fluorescence.

5 25. The assay of claim 1 wherein a voltage imaging plate reader is used to monitor changes in fluorescence.

26. The assay of claim 1 wherein the membrane potential dye is selected from the group consisting of Molecular Devices Membrane Potential Kit (cat#R8034), Di-4-ANEPPS (Pyridinium, 4-(2-(6-(dibutylamino)-2-naphthalenyl)ethenyl)-1-(3-sulfopropyl))-, hydroxide, inner salt), DiSBACC4(2) (bis-(1,2-dibarbituric acid)-trimethine oxanol), DiSBAC4(3) (bis-(1,3-dibarbituric acid)-trimethine oxanol), CC-2-DMPE (Pacific Blue™ 1,2-dietradecanoyl-*sn*-glycerol-3-phosphoethanolamine, triethylammonium salt) and SBF1-AM (1,3-Benzenedicarboxylic acid, 4,4'-[1,4,10-trioxa-7,13-diazacyclopentadecane- 7,13-diylbis(5-methoxy-6,12-benzofurandiyl)]bis-, 15 tetrakis[(acetyloxy)methyl] ester; (Molecular probes).

27. A method for monitoring the activity of an epithelial sodium channel (ENaC) comprising:

providing a test cell transfected with a functional ENaC comprised of 20 alpha (or delta), beta, and gamma ENaC subunits, splice variants, fragments and subunit combinations thereof;

seeding the test cell in the well of a multi-well plate and incubating for a time sufficient to reach at least about 70% confluence;

dye-loading the seeded test cell with a membrane potential dye in the well of the multi-well plate;

contacting the dye-loaded host cell with at least one known ENaC inhibitor at a concentration that at least partially inhibits ENaC function;

5 further contacting the dye-loaded test cell with at least one putative modulating compound and sodium in the well of the multi-well plate; and

monitoring any changes in fluorescence of the membrane potential dye due to modulator/ENaC interactions using a fluorescence plate reader or voltage intensity plate reader.

10 28. The method of claim 27 wherein said test cell is a HEK293 cell.

29. The method of claim 27 wherein said test cell is a HEK293T cell.

30. The method of claim 27 wherein said alpha, beta and gamma subunits are encoded by SEQ ID NO.: 1, 2 and 3 respectively.

15 31. The method of Claim 27 wherein said known ENaC inhibitor is an amiloride derivative.

32. The method of Claim 31 wherein said compound is selected from the group consisting of Phenamil, benzamil, ethylisopropylamiloride; 2', 4' - dimethylbenzamil (DMB), 5-(N-4-chlorobenzyl)-2',4' - dimethylbenzamil (CBDMB); 3', 4' - dichlorobenzamil; 5-(N-methyl-N-guanidinocarbonyl)methyl amiloride, 5-(N,N-hexamethylene)amiloride; 5(N-ethyl-N-isopropyl)amiloride (EIPA); 5-(N-4-chlorobenzyl)- 2', 4' dimethylbenzamil; 2', 4'; -dimethyl 2', 3'-

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benzamil 2', 3'-benzobenzamil; and 5-(N-4-chlorobenzyl)- 2', 4' dimethylbenzamil.

33. The method of Claim 32 wherein said compound is Phenamil.

34. The method of claim 27 wherein said delta, beta and gamma  
5 subunits are encoded by SEQ ID NO.: 7, 2 and 3 respectively.

35. The method of claim 31 wherein the test cell is HEK293.

36. The method of claim 27, wherein the test cell is dye-loaded by  
adding the membrane potential dye to the well of the multi-well plate with the  
test cell seeded therein and incubating for a period of time sufficient to allow  
10 for equilibration of the dye through the membrane of the test cell.

37. The method of claim 36, wherein the membrane potential dye is  
added to the well of the multi-well plate at a concentration of about 2  $\mu$ M to  
about 5  $\mu$ M of the final concentration.

38. The method of claim 27, wherein the membrane potential dye is  
15 selected from the group consisting of Molecular Devices Membrane Potential  
Kit (cat# R8034), Di-4-ANEPPS (Pyridinium, 4-(2-(6-(dibutylamino)-2-  
naphthalenyl)ethenyl) -1-(3-sulfopropyl)-, hydroxide, inner salt), DiSBAC4(2)  
(bis-(1,2-dibarbituric acid)-trimethine oxanol), DiSBAC4(3) (bis-(1,3-  
dibarbituric acid)-trimethine oxanol), CC-2-DMPE (Pacific Blue™ 1,2-  
20 ditetradecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt).  
on SBFI-AM (1,3-Benzenedicarboxylic acid, 4,4'-[1,4,10-trioxa-7,13-  
diazacyclopentadecane- 7,13 -diylbis(5-methoxy-6,12-benzofurandiyl)]bis-,  
tetrakis[(acetyloxy)methyl] ester; (Molecular probes).

39. The method of claim 27, wherein the ENaC is a human ENaC encoded by ENaC subunit DNAs cloned from human kidney cDNA.

40. The method of claim 27, wherein the ENaC is a human ENaC encoded by ENaC subunits DNAs cloned from human lung cDNA.

5 41. The method of claim 27, wherein the ENaC is a human ENaC encoded by ENaC subunits DNAs cloned from human taste cell cDNA.

42. The method of claim 27 wherein the ENaC is selected from the group consisting of: a naturally occurring human ENaC subunit, an alternatively spliced human ENaC subunit, a functional variant thereof and  
10 combinations where the cell expresses alpha, beta and gamma subunits.

43. The method of claim 27 wherein the ENaC comprises alpha (or delta), beta and gamma subunits of a naturally occurring human ENaC, or an alternatively spliced version thereof or combinations thereof.

44. The method of claim 27 wherein the test cell is selected from the  
15 group consisting of MDCK, HEK293, HEK293T, COS, BHK, NIH3T3, Swiss3T3 and CHO cell.

45. The method of claim 27 wherein the test cells are grown to 80% confluence.

46. A method for identifying a salty taste modulating compound  
20 comprising: providing a test cell transfected or transformed with a functional human ENaC; splice variant, chimera or fragment thereof;

seeding the test cell in the well of a multi-well plate and incubating for a time sufficient to reach at least about 70% confluence;

dye-loading the seeded test cell with a membrane potential dye in the well of the multi-well plate;

contacting the test cell with at least one known ENaC inhibitor compound at a concentration whereby ENaC function is at least partially  
5 inhibited;

further contacting the dye-loaded test cell with at least one putative modulatory compound and sodium in the well of the multi-well plate;

monitoring any changes in fluorescence of the membrane potential dye due to modulator/ENaC interactions using a fluorescence plate reader or  
10 voltage intensity plate reader; and

identifying the at least one putative modulator as a salty taste modulating compound based on the monitored changes in fluorescence.

47. The method of Claim 46 wherein the known ENaC inhibitor is an amiloride derivative.

15 48. The method of Claim 47 wherein said compound is selected from the group consisting of Phenamil, benzamil, ethylisopropylamiloride; 2<sup>l</sup>, 4<sup>l</sup> – dimethylbenzamil (DMB), 5-(N-4-chlorobenzyl)-2<sup>l</sup>, 4<sup>l</sup> – dimethylbenzamil (CBDMB); 3<sup>l</sup>, 4<sup>l</sup> – dichlorobenzamil; 5-(N-methyl-N-guanidinocarbonyl)methyl amiloride, 5-(N,N-hexamethylene)amiloride; 5(N-ethyl-N-isopropyl)amiloride  
20 (EIPA); 5-(N-4-chlorobenzyl)- 2<sup>l</sup>, 4<sup>l</sup> dimethylbenzamil; 2<sup>l</sup>, 4<sup>l</sup>; -dimethyl 2<sup>l</sup>, 3<sup>l</sup>- benzamil 2<sup>l</sup>, 3<sup>l</sup>-benzobenzamil; and 5-(N-4-chlorobenzyl)- 2<sup>l</sup>, 4<sup>l</sup> dimethylbenzamil.

49. The method of Claim 48 wherein said inhibitor is Phenamil.



50. The method of claim 46 further comprising evaluating the identified ENaC modulatory compound for effects on salty taste perception.

51. The method of claim 46 wherein said test cell is selected from the group consisting of MDCK, HEK293, HEK2933T, COS, BHK, NIH3T3,  
5 Swiss3T3 and CHO.

52. The method of claim 51 wherein said test cell is an HEK293 cell.

53. The method of claim 52 wherein said test cell is a HEK2933T cell.

54. The method of claim 46 in which the cell is an HEK293 cell.

10 55. The method of claim 54 wherein said HEK293 cell is an HEK293T cell.

56. The method of claim 46 in which a said method is used to identify a compound as one which particularly modulates taste based on a detectable change in fluorescence.

15 57. The method of claim 56 wherein said taste is salty taste.

58. The assay method of claim 46 in which said test cells are seeded on to a well of a multi-well test plate and grown to about 80% confluence.

59. The method of claim 58 wherein said test cells are contacted  
20 with a putative modulator by adding said putative modulator to the well of said multi-well test plate.

60. The method of claim 59 wherein said test cells are loaded with a membrane potential dye that allows for changes in fluorescence to be detected.

61. The method of claim 60 wherein said test cell expresses each of  
5 the alpha, beta and gamma ENaC subunits.

62. The method of claim 61 wherein said subunits are respectively encoded by SEQ ID NO: 1, 2 and 3, or a fragment thereof, or a DNA sequence that hybridizes thereto and encodes a functional hENaC subunit.

63. The method of claim 62 wherein said subunits are encoded by  
10 SEQ ID NO: 1, 2 and 3.

64. The method of claim 46 wherein said test cell expresses hENaC beta, gamma and delta subunits or a fragment or variant thereof.

65. The method of claim 18 wherein said beta, gamma and delta subunits are respectively encoded by SEQ ID NO.: 2, 3 and 7.

15 66. The assay of claim 46, wherein said ENaC subunits all comprise human ENaC subunits cloned from human kidney cDNA.

67. The assay of claim 46, wherein said ENaC subunits all comprise human ENaC subunits cloned from human lung cDNA.

20 68. The assay of claim 46, wherein the ENaC is a human ENaC that is encoded by human ENaC DNA sequences cloned from human taste cell cDNA.

69. The assay of claim 46, wherein the ENaC is comprised of alpha (or delta), beta and gamma subunits and selected from the group consisting

of: a naturally occurring human ENaC, an alternatively spliced human ENaC, a functional variant thereof, or subunit combinations thereof.

70. The assay of claim 46 wherein a fluorescence plate reader is used to monitor changes in fluorescence.

5 71. The assay of claim 46 wherein a voltage imaging plate reader is used to monitor changes in fluorescence.

72. The assay of claim 46 wherein the membrane potential dye is selected from the group consisting of Molecular Devices Membrane Potential Kit (cat#R8034), Di-4-ANEPPS (Pyridinium, 4-(2-(6-(dibutylamino)-2-naphthal-  
10 enyl)ethenyl)-1-(3-sulfopropyl))-, hydroxide, inner salt), DiSBACC4(2) (bis-(1,2-dibarbituric acid)-trimethine oxanol), DiSBAC4(3) (bis-(1,3-dibarbituric acid)-trimethine oxanol), CC-2-DMPE (Pacific Blue™ 1,2-dietradecanoyl-*sn*-glycerol-3-phosphoethanolamine, triethylammonium salt) and SBFI-AM (1,3-Benzene-dicarboxylic acid, 4,4'-[1,4,10-trioxa-7,13-diazacyclopentadecane-  
15 7,13-diylbis(5-methoxy-6,12-benzofurandiyl)]bis-, tetrakis[(acetyloxy)methyl] ester; (Molecular probes).

73. A method for identifying a compound that modulates hENaC comprising;

20 (i) contacting a recombinant mammalian cell that expresses a functional ENaC with a compound known to inhibit ENaC function and further contacting this cell with a candidate compound that putatively modulates an epithelial sodium channel; and

- (ii) determining whether said candidate compound modulates or binds to said hENaC and/or affects the activity of said hENaC.

74. The method of claim 73 wherein said mammalian cell is  
5 selected from the group consisting of MDCK, BHK, HEK293, HEK293T, COS, NIH3T3, Swiss3T3 and CHO.

75. The method of claim 74 wherein said mammalian cell is an HEK293 cell.

76. The method of claim 75 wherein said cell transiently or stably  
10 expresses the alpha (or delta), beta and gamma ENaC subunits.

77. The method of claim 73 wherein said mammalian cell is comprised in a multi-well test plate device.

78. The method of claim 77 wherein said mammalian cell is loaded with a membrane potential dye, contacted with a putative ENaC modulator  
15 and sodium, and change in fluorescence monitored using a voltage intensity plate reader or fluorescence plate reader.

79. The method of claim 78 wherein said mammalian cells are grown to about 80% confluence.

80. The method of claim 79 wherein the membrane potential dyes  
20 are CC2-DMPVE or DISBAC2(3) and ESS-CY4.

81. The method of claim 80 wherein the dye is comprised in a loading buffer.

82. The method of claim 81 wherein after cells are loaded with the dye variation of cell density is evaluated.

83. The method of claim 80 wherein known ENaC inhibitor is an amiloride derivative.

5           84. The method of claim 83 wherein said compound is selected from the group consisting of Phenamil, benazmil, ethylisopropylamiloride; 2<sup>l</sup>, 4<sup>l</sup> – dimethylbenzamil (DMB), 5-(N-4-chlorobenzyl)-2<sup>l</sup>,4<sup>l</sup> – dimethylbenzamil (CBDMB); 3<sup>l</sup>, 4<sup>l</sup> – dichlorobenzamil; 5-(N-methyl-N-guanidinocarbonyl)methyl amiloride, 5-(N,N-hexamethylene)amiloride; 5(N-ethyl-N-isopropyl)amiloride  
10 (EIPA); 5-(N-4-chlorobenzyl)- 2<sup>l</sup>, 4<sup>l</sup> dimethylbenzamil; 2<sup>l</sup>, 4<sup>l</sup>; -dimethyl 2<sup>l</sup>, 3<sup>l</sup>- benzamil 2<sup>l</sup>, 3<sup>l</sup>-benzobenzamil; and 5-(N-4-chlorobenzyl)- 2<sup>l</sup>, 4<sup>l</sup> dimethylbenzamil.

85. The method of claim 84 wherein said compound is Phenamil.

15           86. An oocyte that expresses a functional human ENaC sodium channel comprising  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits encoded by the nucleic acid sequences contained in SEQ ID NOS.:1, 2 and 3 or nucleic acid sequences that hybridize thereto under stringent hybridization conditions or comprising  $\Delta$ ,  $\beta$ , and  $\gamma$  subunits encoded by the nucleic acid sequences contained in SEQ ID NOS:7, 2 and 3.

20           87. The oocyte of Claim 1 which is a mammalian, amphibian, avian or reptilian oocyte.

88. The oocyte of Claim 1 which is frog oocyte.

89. The oocyte of Claim 88 which expresses the nucleic acid sequences contained in SEQ ID NOS. 1, 2, and 3.

90. The oocytes of Claim 88 which expresses the nucleic acid sequences contained SEQ. ID. NOS: 7, 2 and 3.

5 91. A method for identifying a modulator of human ENaC utilizing an oocyte that expresses a functional human ENaC sodium channel with a putative human ENaC modulatory compound, assaying the effect of said compound on sodium transport through the ENaC channel and identifying whether said compound is an ENaC modulator based on its enhancing or  
10 inhibitory effect on sodium transport.

92. The method of Claim 91 which is an electrophysiological assay.

93. The method of Claim 92 wherein said assay is a two-electrode voltage clamp technique.

94. The method of Claim 91 wherein the oocyte is a mammalian,  
15 amphibian, avian or reptilian oocyte.

95. The method of Claim 94 wherein the oocyte is an amphibian oocyte.

96. The method Claim 95 wherein the oocyte is a frog oocyte.

97. The method of Claim 96 wherein the assay is an  
20 electrophysiological assay.

98. The method of Claim 97 wherein said assay is a two-electrode voltage clamp technique.

99. The method of Claim 98 which is used to identify a human ENaC enhancer.

100. The method of Claim 98 which is used to identify a human ENaC inhibitor.

5 101. The method of Claim 96 wherein said frog oocyte expresses the nucleic acid sequences contained in SEQ. ID. NOS: 1, 2 and 3 or SEQ. ID NOS:7, 2 and 3.

102. The method of Claim 97 wherein said oocyte is contacted with an inhibitor of human ENaC prior to contacting with a putative human ENaC  
10 enhancer.

103. The method of Claim 102 wherein said known inhibitor is amiloride or Phenamil.

104. The method of Claim 99 wherein the ability of said putative human ENaC enhancer to specifically enhance human ENaC is evaluated in  
15 at least one additional assay selected from the group consisting of current/voltage (I/V) curve analyses, amiloride competition analyses, and dose-response analyses.

105. The method of Claim 99 which further comprises a negative control using an oocyte that has not been microinjected with human ENaC  
20 cRNAs.

106. The method of Claim 103 wherein the putative human ENaC enhancer and amiloride are co-applied to a human ENaC expressing frog oocyte.

107. The method of Claim 99 wherein said putative modulator is applied at a concentration ranging from around 1nM to about 1mM.

108. The method of Claim 99 wherein said human ENaC enhancer exhibits an enhancement factor of at least 20%.

5        109. The method of Claim 108 wherein said human ENaC enhancer exhibits an enhancement factor of at least 50%.

110. The method of Claim 108 wherein said human ENaC enhancer exhibits an enhancement factor of at least 100%.